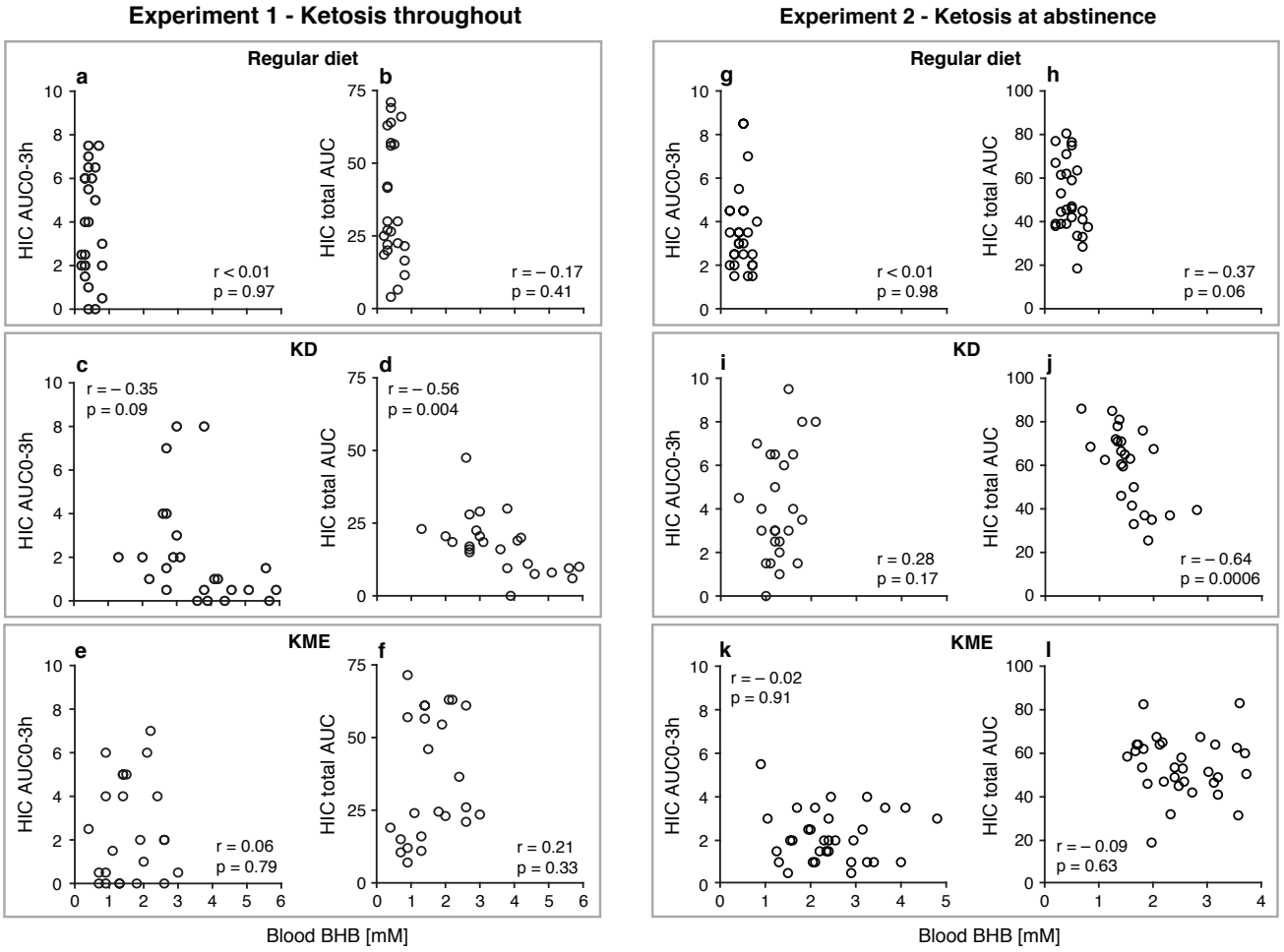
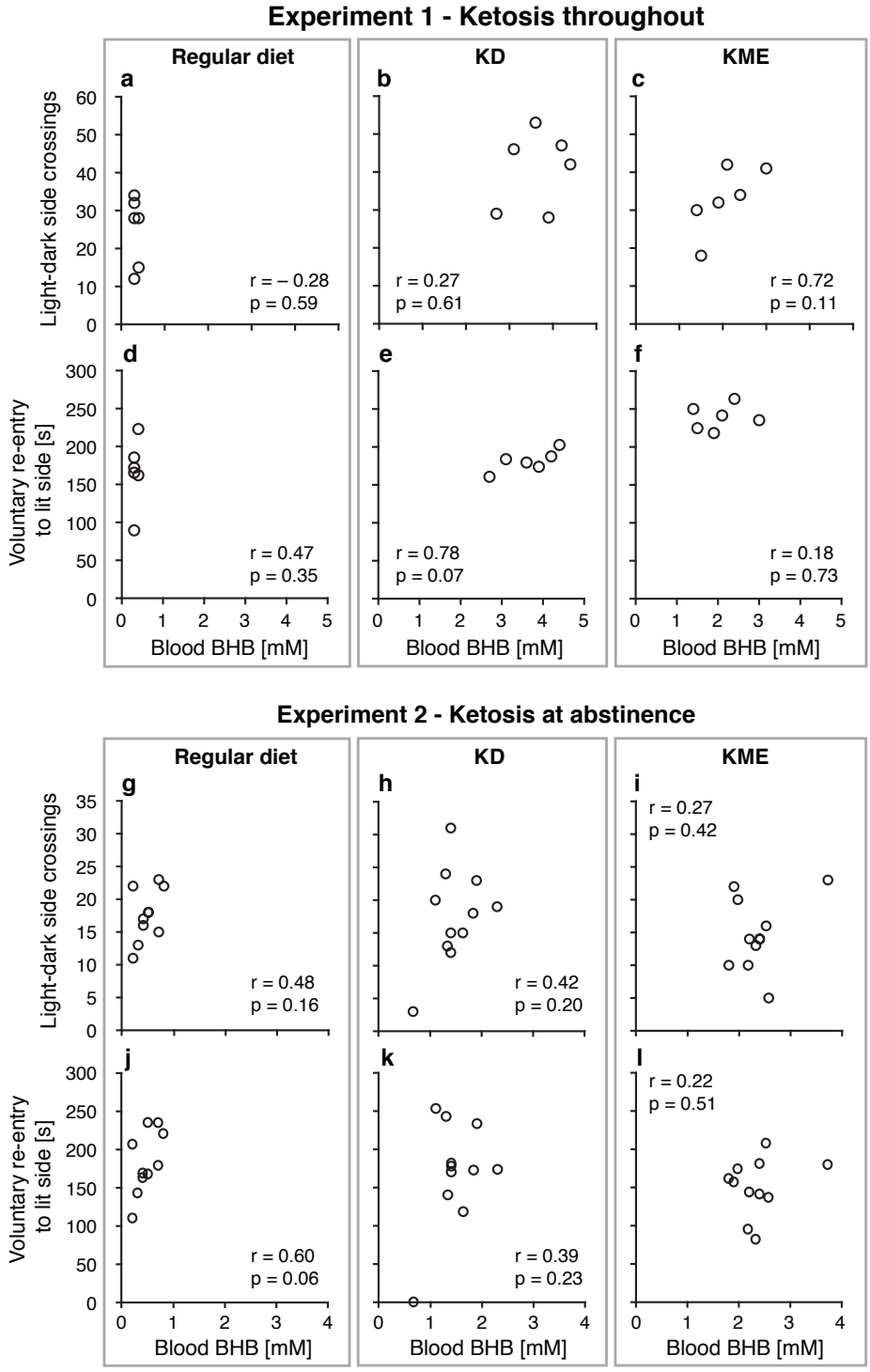
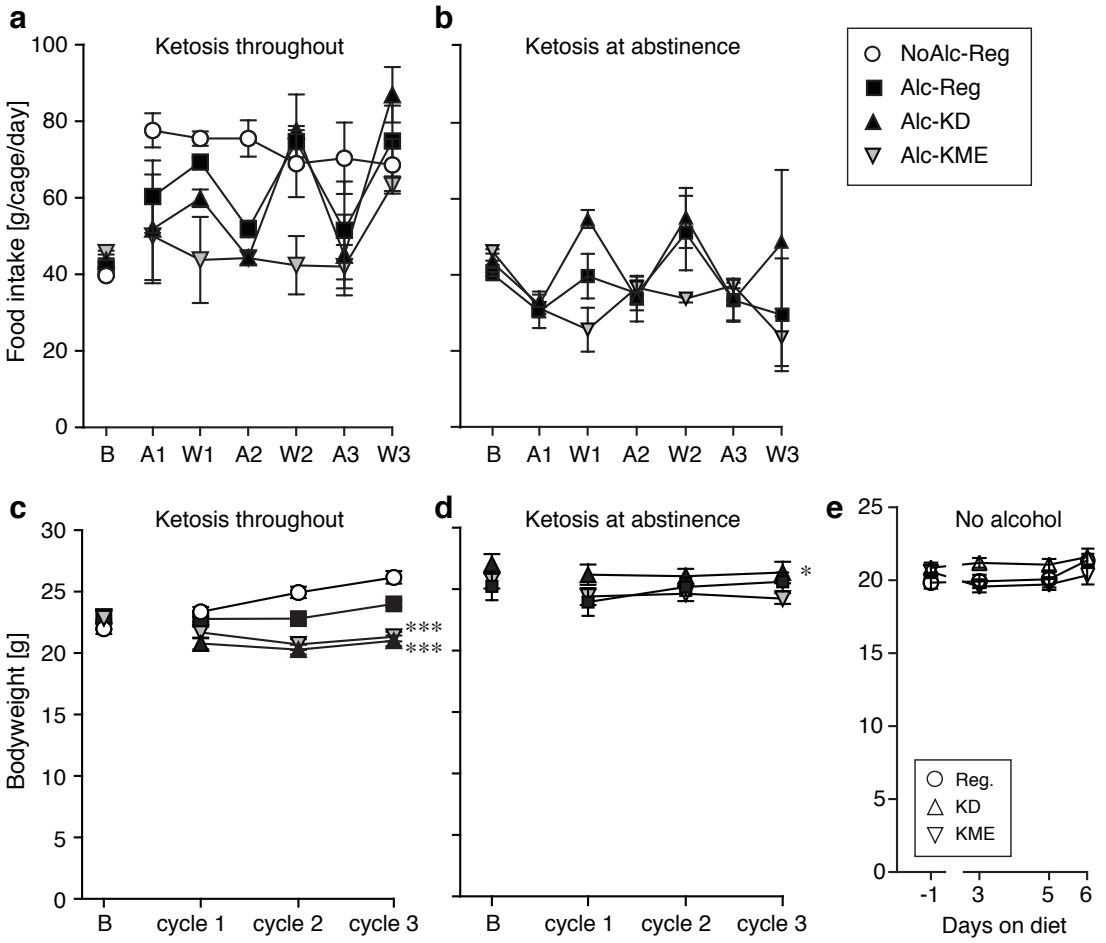


Supplemental Figure S1

HIC scores for the total observation period were negatively correlated with blood BHB the KD group in both experiments, i.e., higher BHB levels were associated with lower seizure scores. (a-f) Experiment 1, (g-l) Experiment 2. Data are individual values of 3h AUC (a, c, e; g, i, k) and total AUC (b, d, f; h, j, l) as a function of blood BHB in the corresponding alcohol exposure/abstinence cycle. Each panel shows correlation coefficient r and significance level p.



Supplemental Figure S2
Behavioral measure in the light-dark transition test did not correlate significantly with blood BHB in Experiment 1 (a-f) or in Experiment 2 (g-l), although in some cases there was a trend towards higher BHB measures correlating with higher scores (interpreted as less anxiety). Data are individual values of light-dark compartment crossings (a-c, g-i) and voluntary re-entry to lit side (d-f, j-l) as a function of mean blood BHB in the third alcohol exposure/abstinence cycle. Each panel shows correlation coefficient r and significance level p.



Supplemental Figure S3
Food intake in g/cage/day (a,b) and bodyweight in g (c-e) in Experiment 1 (a,c, n=12), Experiment 2 (b,d, n=10-12), and no-alcohol controls (e, n=6). *p<0.05, ***p<0.001 vs. alcohol-regular diet. “B”: pre-alcohol baseline, “A1”-“A3”: average of alcohol exposure days for weekly alcohol/abstinence cycles 1, 2, and 3; “W1”-“W3”: withdrawal (forced abstinence) days for cycles 1, 2, and 3. Data are group means ± s.e.m., averaged over the consecutive days in each experimental phase (a-d), or recorded on individual days (e).

Supplemental methods

HIC scoring

Mice were lifted by the tail and observed for 5 seconds, if convulsions were induced, the response was scored and the mouse was gently put back into its cage and observed. If no convulsion was shown, the mouse was gently spun 180 degrees horizontally and observed suspended for 5 seconds more, before being released into its cage.

Possible scores were:

- 0: No convulsion on tail lift, or after gentle 180° spin
- 1: Only facial grimace after gentle 180° spin
- 2: No convulsion when lifted by the tail, but tonic convulsion elicited by gentle 180° spin
- 3: Tonic-clonic convulsion after gentle 180° spin;
- 4: Tonic convulsion when lifted by the tail
- 5: Tonic-clonic convulsion when lifted by the tail, often with onset delayed by as much as to 1 to 2 seconds
- 6: Severe, tonic-clonic convulsion when lifted by the tail, with quick onset and long duration
- 7: Severe, tonic-clonic convulsion elicited before lifting by the tail (spontaneous or elicited by mild environmental stimulus such as lifting the cage).

Score 7 was never observed in this study, and no convulsions lasted past releasing the mouse or necessitated treatment. Nevertheless, diazepam was kept immediately available in the test room as pre-filled syringes for “rescue” if needed.

Loss of righting reflex

Loss of righting reflex was recorded 2 minutes after alcohol administration, then, every 10 minutes until righting reflex was recovered in two consecutive tests. Testing was done by placing the mouse in the supine position in a V-shaped holder and observing for the ability to right itself onto its legs. Righting (or lack of righting) was tested twice consecutively at each time point. Loss of righting reflex was defined as the inability to right itself twice within 30 seconds, recovery was defined as the mouse righting itself twice within 30 seconds. Loss of righting reflex duration was the time between loosing and recovering the righting reflex.

Operant procedure, oral alcohol self-administration

Mice were first introduced to alcohol drinking using a drinking in the dark procedure in the home cage (Rhodes et al. 2005). Water bottles were replaced with unsweetened 20% alcohol 4 h/day (beginning 3 h into the dark phase) Tuesday-Friday. The following Monday, mice were allowed to acquire nose-poking under a fixed ratio 1 timeout 20 s schedule of reinforcement in daily 2h sessions, first with a non-sweet liquid food (Nutridrink, Vanilla flavor, Nutricia Denmark) as the reinforcer. When at least 20 reinforcers were earned per session for two sessions (typically 2-4 sessions), food was replaced with the oil-water emulsion Calogen (unflavored, Nutricia), then 10% alcohol in Calogen, then 20% alcohol in Calogen, for at least two sessions each and until at least 15 reinforcers were earned (typically 2 sessions each). Liquid reinforcers were 30µl. Finally, responses were reinforced with 20% alcohol in water for a minimum of five baseline sessions and throughout testing. Mice met baseline criteria when they maintained ≥70% active responses and a ≥15/session reinforcer average over five consecutive sessions, with one session >10 but <15 reinforcers acceptable if followed by at least two sessions ≥15. 15 reinforcers/session equates to 3.0 g/kg alcohol for a 24g mouse. A limit of 50 alcohol reinforcers per session (9.9 g/kg in a 24g mouse) was implemented to prevent overdose (Sørensen et al. 2016). Alcohol intake as g/kg/session was calculated for each mouse, adjusted for any alcohol solution left unconsumed after the first two groups (typically none or very little). Food-reinforced mice were trained with Nutridrink only, to criteria of at least 25 reinforcers per session for three consecutive sessions.

Diet ingredients

KetoCal (a nutritionally complete, ketogenic formula with a 4:1 fat:(carbohydrate + protein) ratio; Nutricia, Denmark); black currant flavor Nutridrink juice style (a carbohydrate and whey protein-based,

vitamin-mineral enriched nutrition drink; Nutricia); casein protein powder, fibers (cellulose, xantham gum, psyllum husk); filtered tap water.

Rhodes, J. S., Best, K., Belknap, J. K., Finn, D. A., Crabbe, J. C., 2005. Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiology and Behavior 84, 53-63.

Sørensen, G., Caine, S. B., Thomsen, M., 2016. Effects of the GLP-1 Agonist Exendin-4 on Intravenous Ethanol Self-Administration in Mice. Alcoholism: Clinical and Experimental Research 40, 2247-2252.